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AMXFD D/A ltr, 9 Feb 1972

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AD 836626

TRANSLATION NO. 1136

DATE: 4 Aug 1964

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

CHRONOPHOTOMETRIC MEASUREMENT OF CHEMICAL LUMINESCENCE
IN THE HYDRAZIDE OF TRI-AMINOPHTHALIC ACID (LUMINOL)

(A report delivered at the 9th National Congress on Chemistry, held jointly with Swiss Chemistry Society at Naples, 27 May-2 June 1962, by Enrico Bovalini and Marcello Pizzetti, and published in the Annali di Chimica Vol. 53, No. 8/9, 1963, pp 1103-1109.)

SUMMARY -- Research was conducted into the variations in intensity and duration of the emission of light which occurs in reactions of the hydrazide of tri-aminophthalic acid (luminol) in the presence of hydrogen peroxide and iron compounds. Suitable experimental apparatus for taking such measurements were devised, and the results obtained show, in some areas, a significant proportional relationship between the maximum light intensity given off and the concentration of peroxide. A direct relationship was also observed between the maximum light intensity and the concentration of hemin when hemin was used as a catalyzing iron compound.

The literature contains several studies, ranging from 1928 (1) to the present, on the phenomenon of chemical luminescence shown by certain reactions of substances such as the hydrazides of phthalic acid. In all these studies, attention is focussed on the chemical and physical aspects of the phenomenon in the presence of various reagents, particularly of hydrogen peroxide, and of catalysts that act either on its rate of decomposition or on the speed of formation or decomposition of possible peroxide compounds, with the emission of light occurring at some stage of the process. Various interpretations (2,4) have been offered, and a number of theories (5, 6, 7, 11) suggested to account for it. Particular attention was given to the phenomenon as produced by the peroxides by H. D. K. Drew and R. F. Garwood (8).

The luminescent process can be analyzed by controlled variation of the amount of hydrogen peroxide and other peroxides involved in the reaction. (9, 10-12). Other substances which produce chemical luminescence were used, such as lucigenina (13) rimifon (14) etc. Catalysts used, in addition to iron compounds, were compounds of other metals such as cobalt, manganese, copper, which yielded interesting results (15, 16).

The more important analytical uses referred to above were

those connected with the qualitative identification of traces of peroxides, the attempts at quantitative analysis, and the use as an index of acidity, since light emission is a function of the pH. The phenomenon is usually first perceptible at about pH 7, and increases towards alkalinity. For luminol (hydrazide of ortho-aminophthalic acid, or, in the quinonic form corresponding to it, 5-amino phthalazine-1,4-dione), the optimum pH is about 10.3.

In order to describe these analytical applications adequately, and at the same time to contribute to our knowledge of the mechanism of chemical luminescence, we determined to study the conditions governing intensity and duration of the phenomenon in relation to the characteristics of the reagents, so as to assign to each of them a rating as stoichiometric or catalytic. The reaction between peroxides and luminol occurs with a marked emission of light in the presence of hemin at pH 10.3, and lasts for periods varying from several seconds to twenty or thirty minutes, according to the concentration of H_2O_2 . Some of our initial experiments seemed to indicate that hemin, considered a catalyst for the phenomenon, was apparently stoichiometric in its behavior in relation to the intensity of the light emission and its length. We say apparently because, according to H. Ojima and K. Sone (17), the maximum intensity of light emission (the peak) can be taken as a measurement of catalytic activity. This in turn made it worth while to study the kinetics of the phenomenon by means of measurements of the intensity and duration of light emission, with alternating variations in the concentrations of all our reagents, so as to establish which of them exerted markedly stoichiometrical or catalytical effects.

EXPERIMENTAL PORTION

Equipment. - The apparatus used for studying this reaction consisted of two parts: an instrument in which a suitable container (a beaker of 50 cc) is placed in a completely sealed thermostatic chamber, equipped with a cover having an opening to admit the mechanical agitator and another for introducing reagents by means of a special burette. At the bottom, the thermostatic chamber has another opening, which contains a cathode ray cell, type 90AG, with a sensitivity (Va-85V) 130 mA/1', powered by a suitably stabilized feed circuit. The output of the cell is sent to a Kipp recorder, of the Micrograph B3 type, which has the following characteristics: maximum sensitivity, 0.1 mA-0.05 mV; paper speed from 40 to 960 mm/min.

In the container we placed measured amounts of solution with a known concentration of luminol and hemin or other similar reagents. Water, heated to 20°C, was circulated in the insulating jacket around the container. Through the burette, we then introduced measured

amounts in known concentrations of solutions of H_2O_2 or other peroxides, taking care to perform the operation as quickly as possible, sometimes even applying a slight pressure on the upper end of the burette.

The evaluation of the response interval of the photoelectric cell and the recorder (the period of the instrument) can be performed once and for all with a light source placed inside the container and lighted simultaneously with the recorder, so as to check the time lag in recording. We found that when the phenomenon lasted more than five or six seconds, this hysteresis in the apparatus was negligible.

This apparatus gave us certain curves which, with the precision of the corresponding parameters (paper speed, response of the photoelectric cell and sensitivity of the recorder), indicate, by the height of the peaks (y) the intensity of the light emitted, and with the distance between the time, 0, and the point at which $y = 0$ (the end of the phenomenon) on the (x) co-ordinate, the duration of the phenomenon.

Solutions used. -- Solution of luminol 0.005M: 1.1629 of the hydrochloride of the hydrazide of tri-aminophthalic acid are dissolved and brought to 1 l in a solution of Na_2CO_3 0.2N.

Solution of hemin 2.10^{-4} M: 0.1126 of hemin hydrochloride are dissolved and brought to 1 l in a solution of Na_2CO_3 0.2N.

Solutions of hydrogen peroxide: solutions of 0.1M to 0.001M were prepared immediately before use, by diluting a 36% solution of H_2O_2 , controlling and checking its strength with a 0.1N solution of $KMnO_4$.

Performing the experiment. - We introduced into the container known quantities of solutions of luminol and hemin, and enough Na_2CO_3 0.2N solution to bring the total volume to 20cc and to an approximate pH of 10.3 (± 0.05). With contacts made, the agitator on, and the temperature set at $20^\circ C$, we started the recording device and, as quickly as possible, introduced 1cc of H_2O_2 in a known concentration. In this connection, we note that we always used the same volume of H_2O_2 , at varying levels of concentration, so as to make our measurements on the same volume at all times.

Figures 1 and 2 show some of the significant curves obtained with the procedure outlined above. Table 1 shows data from several experiments in which we varied the concentration of hydrogen peroxide, keeping the concentrations of hemin and luminol constant, and others in which we varied the hemin concentration, keeping the luminol peroxide concentrations constant.

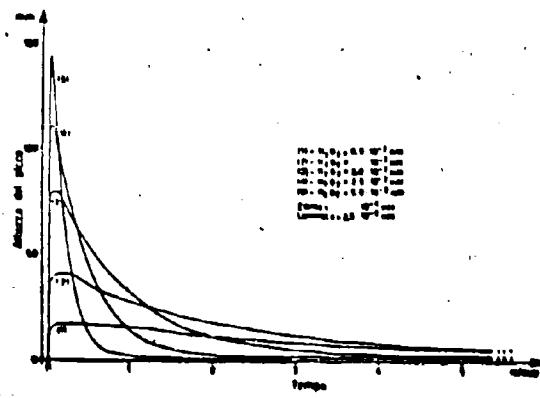


Figure 1

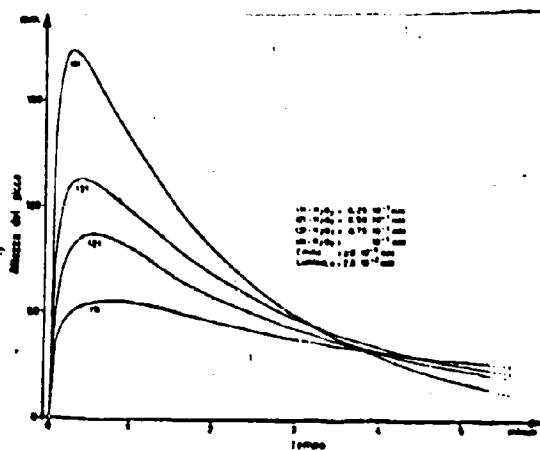
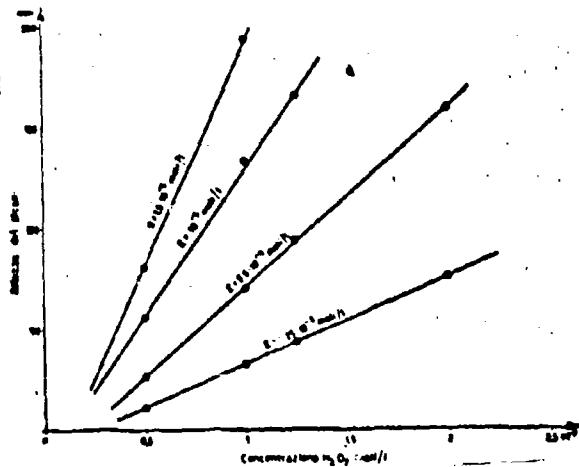


Figure 2



Altezza del picco = Height of the peak
 Concentration of H_2O_2 1 mole

Figure 3

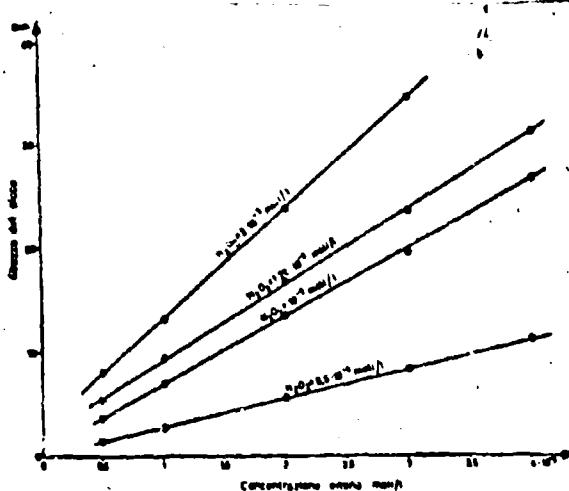
Figure 3 shows the absolute linearity of the relation between peak height and H_2O_2 concentrations over a considerable interval, which refers to the first five experiments on the table. This makes it possible to calculate quantities of H_2O_2 as small as 1 ppm with remarkable precision.

Experiments 6 to 11 are shown on figure 4, and indicate the variations we found in the intensity of light emission (height of the peaks) as a function of the variation in hemin concentration per fixed quantity of peroxide. The effects of this variation are quite evident; as the concentration of hemin increases, you get a marked increase in the quantity of light instantaneously emitted: at the same time, you get an increase in the duration of the phenomenon. Figure 5 reproduces the recorded tracks (chronophotograms) of the phenomenon. Note also the direct proportion between the height of the peak and hemin concentration in every concentration of H_2O_2 tested. This allows identification of as little as 2 ppm of hemin.

Table 1

EXPERIMENTS PERFORMED ON 20CC OF SOLUTION AT pH 10.3
IN THE PRESENCE OF $2.5 \cdot 10^{-2}$ mM OF LUMIROL

Exp.	H_2O_2 $\times 10^{-3}$ mM	Hemin $\times 10^{-3}$ mM	Height of peak in mm	Exp.	H_2O_2 $\times 10^{-3}$ mM	Hemin $\times 10^{-3}$ mM	Height of Peak in mm
1	0,5	0,5	25	6	0,5	0,5	34
2	1	0,5	70	7	0,5	1	68
3	1,5	0,5	117	8	0,5	1,5	101
4	2	0,5	158	9	0,5	2	122
5	2,5	0,5	215	10	0,5	3	184
—	—	—	—	11	0,5	6	269



Altezza del picco = Height of the peak
Concentrazione emina moli/l = Hemin concentration 1 mole

Figure 4

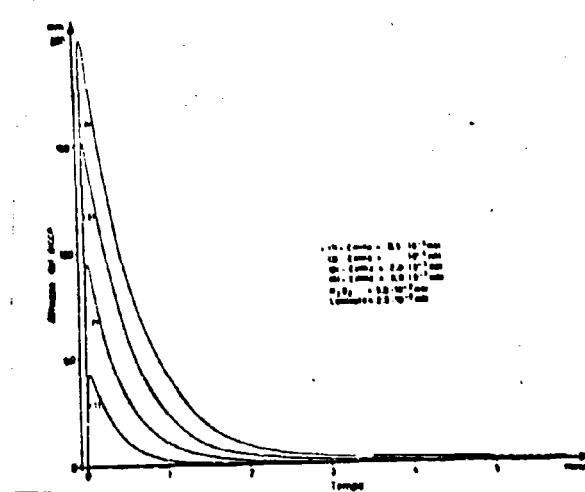


Figure 5

No interpretation of this hemin behavior can be attempted on the basis of so small a number of experiments as these; our only aim was to ascertain the possible applicability of the phenomenon and of the apparatus described in analysis. A working hypothesis, to be checked by further experiment, could be made to serve as an interpretation of the proportional relationship that appears on figures 4 and 5 between the height of the peaks (showing maximum light intensity) and hemin concentration. This might lead one to surmise that hemin's behavior tends more towards the stoichiometric than the catalytic. First, however, there must be experimental establishment of the degree to which the height of the peak (maximum intensity of light emission) is proportional to the quantity of the luminescent product formed, to the ratio between its speed of formation and its rate of decomposition, in which case the height of the peak could also be used as a measure of catalytic activity.

CONCLUSIONS

The experimental apparatus described in this report is well adapted to the quantitative study of the phenomenon of chemical luminescence in luminol. It enabled us to note the intervals and the conditions under which the emission of light is proportional to the concentration of peroxide and the concentration of iron compounds, making possible the analytical use of chronophotometric measurements.